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A New Era in Membrane Channel Biology

Essay

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Although Nobel Prize winners are annually announced with much anticipation, this year's award of the Nobel Prize in Chemistry to Peter Agre and Roderick MacKinnon came as no surprise to the community of structural biologists. We have been joyfully benefiting from the fruits of their labors for some time now.

In what could be dubbed a celebration of achievement in membrane channel studies, Agre has been recognized for his discovery and characterization of the aquaporin family of water channels, and MacKinnon for determining the first high-resolution structures of ion channels. Collectively, their groundbreaking work has made it possible to understand the channel-based mechanisms facilitating the rapid movement of water and ions across cell membranes.

Deciding that he would rather pursue a research career than practice medicine, Roderick MacKinnon followed the postdoctoral route and returned to the laboratory of Christopher Miller at Brandeis University where he honed his skills in ion channel research. Early on, MacKinnon focused his attentions on the potassium channel and the use of physiological, biochemical and molecular biological techniques to explore the influence of various residues on the behavior of the channel. While such experiments teased out bits and pieces of the mechanism regulating ion selectivity, MacKinnon became convinced that "the big picture" would emerge from the molecular details that only a high resolution structure could provide. It was then that MacKinnon made his second major career decision: he decided to direct his energies exclusively into the methods of X-ray crystallography and on obtaining a high-resolution structure of a potassium channel.

During this period other groups were also attempting to crack the ion channel structure problem through either electron or X-ray crystallographic techniques. A discovery that enabled the MacKinnon group to get a handle on the problem was the identification of a robust potassium channel (KcsA) from *Streptomyces lividans*. This channel could be readily solubilized with detergent in a functionally stable state and its overexpression when cloned into an *E.coli* expression vector allowed for crystallization trials to be conducted on an array of modified molecules until a form amenable to crystallization could be identified.

Within two years of embarking on his quest, MacKinnon caught both the ion channel and protein structure communities completely off guard by publishing the first structure of an ion channel. In this structure of the KcsA channel, the simple but elegant solution to achieving high ion selectivity while facilitating rapid potassium ion transport rates was revealed. Four monomers containing two transmembrane and one short pore-based α helix each come together to form one functional channel. Half of the channel pore is wide enough to accommodate a solvated potassium ion while the other half forms a narrow selectivity filter arranged so that only a cation with the ionic radius of a potassium ion can readily proceed through it. Surprisingly, this functionally critical selectivity filter was found to be made up solely of the exposed carbonyl groups from four precisely positioned loop segments; the structure of the region is so well maintained that the slightly smaller ionic radius of the sodium ion, for example, is too small to energetically support the replacement of the ion's hydrating waters for easy entry into the selectivity filter.

In the short span of time since then, MacKinnon has followed up this achievement with a remarkable string of ion channel structures elucidating higher resolution details of potassium transport, the general mechanics of channel gating, inward rectification, chloride selective transport, and voltage-dependent gating. This latter work on the structure of the voltage-dependent potassium channel deserves special note and has revealed the beautifully efficient molecular mechanism behind voltage gating providing the molecular details needed to solve long standing electrophysiological questions.

Following a brief period after obtaining his Ph.D., Peter Agre returned to Johns Hopkins as a hematologist. His early research efforts were centered on studies of Rh factor. In the process of isolating and purifying the Rh blood group antigen 32 kDa subunit from red cell membranes, Agre and coworkers encountered a 28 kDa protein that copurified with it. At first it was thought to be a proteolytic degradation product of the Rh protein. Further investigation determined that it was a red cell membrane protein previously undetected due to its poor Coomassie blue staining properties. Interestingly and importantly, this protein was found to be present in high copy number and could be readily purified.

Although it represented a significant change in the direction of his research program, Agre decided that this unidentified protein would become the group's new focus of attention. Following a hunch that this new integral membrane protein might be the long sought after water channel, Agre and coworkers worked intensively to develop an assay for assessing water transport to evaluate this hypothesis. These efforts culminated in an innovative series of experiments assessing the osmotic behavior of *Xenopus* oocytes expressing this protein. When placed in hypotonic media following mRNA microinjection of the functionally undefined protein, these oocytes dramatically swelled until bursting while control oocytes did not. These results definitively demonstrated that this protein, then termed CHIP28 and now designated AQP1, was indeed a water channel.

The initial groundbreaking discoveries of the Agre group prompted an extensive series of biochemical and biophysical studies at laboratories throughout the world.

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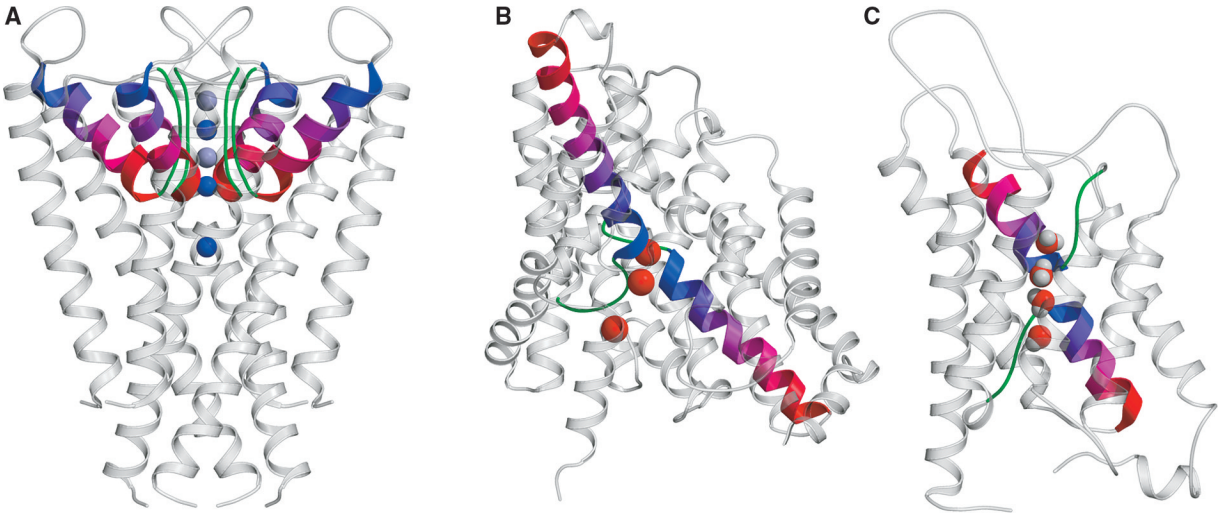

Figure 1. Helix-Loop Motifs of the Ion and Water Channel Selectivity Filters

Colored regions of the (A) potassium channel, (B) chloride channel, and (C) AQP1 water channel ribbon diagrams highlight the helix-loop motifs (red side of the helices indicates the negative end of the helix dipole while blue indicates the positive end; the loop regions of these motifs are shown in green) and their respective solutes (potassium ions by blue and waters by light blue spheres in [A], chloride ions by red spheres in [B] and waters by red/white molecules in [C]). The depiction of two potassium ions and two waters in the selectivity filter of (A) represents one of two possible configurations. In the other configuration the position of the waters and potassium ions are inverted. Figure prepared by Bong-Gyoon Han.

Today, in large part due to the efforts of Agre and collaborators, 11 eukaryotic members of the aquaporin family have been identified and characterized. An international effort arose to determine the molecular structure of a water channel. Several groups made significant strides toward this goal using electron and X-ray crystallographic methodologies. The initial density maps of AQP1 were obtained by electron crystallography studies. Structural models developed from density maps at about 4 Å resolution revealed the channel's secondary structure topography, six transmembrane and two short pore-based α helices. Based on this information, Agre and his collaborators proposed that helix dipole partial charges provided by the two short helices positioned inside the pore would be important in disrupting proton transport through the channel. Determination of the high resolution structures of the glycerol transporter GlpF and AQP1 by X-ray crystallography provided the atomic details of the selectivity filter and channel bound waters required to explain the molecular basis of water selectivity at high throughput rates. Subsequent molecular dynamics studies based on these structures provided unique quantitative insight into the energetics and time-dependent behavior of waters traversing the channel.

From a structural biology point of view, these ion and water channels utilize several interesting common structural themes. Contrary to the notion that charged residues are essential in establishing ion selectivity, the ion channel structures currently available indicate that partial charges, provided by sources such as polar side chains, α carbon backbone amide and carbonyl groups, and helix dipoles, are the predominant elements needed to shape ion selectivity. Such "weaker" solute binding strategies achieve rapid transport rates by minimizing the energy gained in binding to the selectivity filter. In the case of the water channels, this is achieved by placing relatively low numbers of water-coordinating partial charge groups strategically along the lengths of their predominately hydrophobic selectivity filters.

Another interesting observation is that the longest selectivity filter region within the group of membrane channel structures determined to date turns out to be no more than about one-half the overall length of the channel; in the case of the ClC chloride channel, the selectivity filter is only about 12 Å long. The relatively short lengths of these selectivity filters contribute substantially to lowering the overall energy cost of transporting desired solutes, once again helping to increase the rate at which ions and waters can be carried across cell membranes.

A striking feature shared by both ion and water channel structures is the use of pore-localized helix-loop motifs to establish solute coordinating regions within their respective selectivity filters (Figure 1). The potassium and chloride channels, the GlpF bacterial aquaglyceroporin homolog and the AQP1 aquaporin structures have all been found to utilize some combination of these motifs. In each of these channels, the α carbon backbones of helix-loop motif loop regions provide partial charge groups for coordinating ions or water. The α helices of these motifs, oriented to place either the positive or negative ends of their helix dipoles into the selectivity filter, also provide a means of ion selectivity.

A new era of membrane channel biology has been ushered in through the exciting accomplishments of Peter Agre and Roderick MacKinnon. Their research and discoveries have been pivotal in shaping our molecular level understanding of ion and water transport mechanisms. This knowledge, in turn, has already begun to provide insight into the molecular basis of fundamental biological processes and associated diseases, and the structural understanding essential for the development of future therapeutics.